STUDIES OF GRAFT TRANSFER OF HERITABLE TRAITS IN RAPESEED (*Brassica napus* L.)AND MUSTARD (*B. juncea* L. Czern and Coss.)

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ABSTRACT

A study of graft transfer of qualitative traits in *B. napus* and *B. juncea* indicated that heritable transfer occurred for glucosinolate content and anthocyanin pigmentation, but not for male sterility, or erucic acid content. No visible trait transfers were observed in reciprocal grafts between the two species.

INTRODUCTION

It is widely held that grafting does not result in the movement of heritable genetic traits between stock and scion. However, data from a number of studies indicate that indeed such transfers do occur (Yagishita, 1961 a, b; Yagishita et al. 1985; Yanishita and Hirata, 1986, 1987; Ohta, 1975 a, b; 1977; Hirata, 1979, 1980, 1986; Hirata et al. 1990). Hirata and Yanishita (1989) suggested that the mechanism of such changes is likely through the transmission of DNA from degraded cells in the stock, to scion germ cells via the vascular system, and the subsequent integration of DNA into chromosomes. In support of this idea, Ohta (1991) reported on microhistological analysis of the stock in red pepper which indicated chromatin masses of various sizes and shapes were moving through cell walls and intercallular spaces of the scion from the lignifying and dying cells of the stock graft wound. Such supportive evidence spurred an interest in the present study which reports the results of graft transfer experiments in rapeseed and mustard.

EXPERIMENTAL

Material and methods

Grafting experiments were conducted with the following species and trait conbinations:

B. napus A reciprocal graft was made using the wedge grafting technique on the cytoplasmic male sterile (Polima) line Xiangai A as stock, and the male fertile line, cv. Xiangyou 11 as scion, carrying a fertility restoring gene. All grafts were made at the rosette and peduncle stages for both stock and scion. A similar reciprocal grafting experiment to the above was conducted using the low glucosinolate, low erucic acid cv. Xiangyou 11 as stock and normal rapeseed, cv. Chuonyou 9 as scion.

B. juncea A graft was made between cv. Zijie (stock) with heavy purple anthocyanin pigmentation, and cv. Taojingjie (scion) with normal green pigmentation. The graft was made at the rosette stage for both stock and scion.

B. napus and *B. juncea* A reciprocal graft was made between *B. napus* cv. Xiangyou 11 (stock) and *B. juncea* cv. Taojingjie (scion) with the stock at the rosette stage, and the scion at the peduncle stage.

In each of the above experimants, grafting was performed on potted plants grown outdoors which had derived from selfpollination of the parent lines for at least 6 previous generations. Each pot contained two plants, and each experiment (excluding reciprocals) included approximately 20 pots. At flowering, all scions (V_0) were self-pollinated by placing a glacine bag over the racemes. Self- pollinated seeds from each scion were bulked to from a (V_1)population for each experiment which was planted in the field and evaluated for stock/scion trait transfer.

Seed quality analysis of glucosinolates and erucic acid were performed using the techniques described by McGregor (1990) and Thies (1971).

Results

The male sterile (stock)/male fertile (scion) grafting experiments are done. Of the 25 successful grafts at the rosette stage where the stock was male sterile and the scion male fertile, all resulted in male fertile scions, and male fertile V_1 progeny while the reciprocal graft produced 19 male sterile scions only. A similar pattern was recorded for grafts performed at the peduncle stage. The data show that for cytoplasmic male sterility, no discernable genetic exchanges have occured between stock and scion. This result in not unexpected since for such exchanges to happen, movement of whole organelles through cell walls would have to occur, because cytoplasmic male sterility is determined by mitchondrial elements, Kaul (1988). When the scion was male sterile, there should be opportunity for restoration of fertility to occur since fertility restoration in Polima cytoplasm is controlled by a single nuclear dominant gene present in the stock (Fu, 1981; Fang and McVetty, 1989).

The results of reciprocal grafting between the low glucosinolate, low erucic acid line (double low) and the high glucosinolate, high erucic acid line (double high) were similar whether grafting occurred at the rosette or peduncle stage (Table 1). In both cases, there were no detectable differences in erucic

acid content whether the stock or scion was double low or double high. On the other hand, glucosinolate content appeared to increase in the double low scions grafted onto a double high stock, irrespective of whether the graft occurred at the rosette or peduncle stages. Since erucic acid is controlled by 2 major codominant genes (Harvey and Downey, 1964), the chances of obtaining trait changes for this chracter would be less likely than perhaps with glucosinolate content, since this trait is controlled by as many as 4 or 5 independent genes with additive effects (Kondra, Z. P. and Stefansson, B. R. 1970).

TAVLE 1. The effects of reciprocal graft on graft transfer of low erucia acid and low glucosinolate levels in seed of *B. napus*

Grafting stage		No. of plants	Erucic acid (%, X±SE)	Glucosinolate (umol/g, X±SE)
Rosette	1. Stock(double high)		46.70	123.30
	Scion(double low)		0.35	26.8
	V_0	15	0.23±3.80	38.2±8.15
	V_1	34	0.34±5.51	36.6±1.23
	2. Stock(double low)		0.35	26.8
	Scion(double high)		46.70	123.30
	V_0	21	44.80±7.56	121.1±13.3
	V_1	38	47.20±5.31	128.0±11.1
Peduncle	1. Stock(double high)		46.70	123.30
	Scion(double low)		0.35	26.8
	V_0	18	0.38±5.30	46.50±12.67
	V_1	33	0.24±3.15	41.10±10.93
	2. Stock(double low)		0.35	26.80
	Scion(double high)		46.70	123.30
	V_0	23	46.00±7.26	119.21±8.75
	V_1	34	48.30±6.37	127.33±7.23

In the grafting experiment between two *B. juncea* lines, cv. Zijie (stock) with heavy purple pigmentation, and cv. Taojingjie (scion) with nomal green pigmentation, 29 successful grafts plants were obtained. Among the V_0 plants, 9 had light purple pigmentation while the remainder were green in color. The self pollinated progenies from these plants produced 46 plants of which 34 were green in color and 12 were of light purple pigmentation.

The reciprocal graft between *B. juncea* and *B. napus* did not show any visual differences (V_0) when either species was grafted at the rosette or peduncle stages. Likewise, there were no differences in the (V_1) generation . Since only visual traits were noted in these observations, the presence of induced biochemical or physiological changes is not precluded.

Discussion

This study has shown that both anthocyanin pigmentation, and glucosinolate

content can be genetically altered through rapeseed stock-to-scion grafts. However, male sterility, erucic acid content, and interspecific transfer apparently are not effected by grafting. According to previous studies, anthocyanin pigmentation in *B. juncea* and glucosinolate content in *B. napus* are controlled by 2 and 5 gene lairs respectively (Liu, 1985; Kondra and Stefansson , 1970). In the case of glucosinolate content, the effects of these genes are additive, with the cumulative presence of each recessive allele effectively reducing glucosinolate levels in a stepwise fashion, or alternatively, an increase in glucosinolate levels is associated with stepwise addition of dominant alleles. It is therefor, possible in the present study that only one or two loci were effected by the graft tranfers, hence, the slight reduction in glucosinolates. Since anthocyanin pigmentation is under simple gentic control, graft transfer of this trait might be expected te be easier than with more complex traits, such as cytoplasmic male sterility which may require whole organelle transfer. Anthocyanin graft transfers have been the subject of interspecific chimeras produced by grafting the redpigmented cultivar, Ruby Ball of *B. oleracea* with the green pigmenteed cultivar, Komatsuna of *B. campestris* (Noguchi et al. 1992). In transverse sections of leaves and petioles from Komatsuna scions on Ruby Red stocks, cells containing anthocyanin pigments were observed in the mixed state indicating that chimeric tissues were of a complex or sectoral-peripheral type. Even the green tissues showed electrophoretically separable isozyme patterns of both parents. Unfortunately, plants were not regenerated from these tissues, but the evidence seems clear that heritable changes were graft-transferred in this case.

Although graft transmissible influences have been reported on fatty acid composition in soybean (Carver at el. 1987), no effects on erucic acid levels were noted in the present study. Our results are in agreement with those of Kraling and Robbelen (1991) who found no detectable influences of grafting on erucic acid levels in rapeseed.

The chimeric nature of graft transfers suggests that seed samples be collected on a raceme, or perhaps single pod basis rather than from a single plant bulked sample such as was used in the present study. It is possible that had sub samples been taken from single plants, additional transfers may have been detected. This problem should be the subject of farther study.

REFERENCES

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